STUDIES ON ANTIMICROBIAL AND ANTIBIOFILM ACTIVITIES OF ACTINOMYCETES AGAINST URO PATHOGENS

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1. Abstract

Urinary tract infection (UTIs) are becoming more challenging in the recent era due to their high recurrence rates and resistance to frontline antibiotics. Uropathogenic bacteria and mostly *E.coli* accounts for the 90% of all UTIs. The Uropathogens possess various virulence factors that contributes for their effective colonization, higher persistence and pathogenesis of the disease. Biofilm formation ability of these pathogens is mainly linked to nosocomial infection and particularly catheter associated UTIs. Current microbial inhibition strategies are based planktonic bacterial physiology and known to have limited efficiency on the growth of biofilm communities. This problem is further intensified by the emergence of increasingly resistant strains. At present more attention is given to isolation of non-toxic bioactive compounds capable of inhibiting biofilms. One of the such attractive bioresources is actinomycete based novel bioactive molecules. Actinomycetes are known for their potential to produce antibiotics, enzymes, enzyme inhibitors and other pharmaceutical agents. Several reports revealed that many actinomycetes produce antibiofilm agents against different pathogenic organisms. However, the efficiency of these antimicrobial agents is currently decreasing due to emergence of multi drug resistant pathogens. In addition to this very few reports are found against uropathogenic biofilm forming organism. Therefore, it is important to search for alternative therapeutics to control biofilm associated uropathogenic infections. In the present study we propose to characterize the bioactive metabolites produced by actinomycetes and examine it's role in inhibition or dispersion of biofilms formed by uropathogens.

2. Introduction

Urinary tract infection (UTI) is the second most common microbial infection in humans (Foxman, 2014., Franceskoet al., 2016), affecting around 150 million people each year globally. It poses a heavy economic and public health burden with estimated cost in around six billion dollars for treatment and healthcare (Stamm and Norrby, 2001).Bacterial urinary tract infections

affect all age groups including young women, children, and the elderly. It is estimated that approximately 40% of women have had a UTI at some time in their lives. They may occurin any part of the urinary tract and being more common in diabetic patients. Diabetic cystopathy and micro-vascular diseases may cause changes in host defense mechanism of kidney leading to moreprevalence of UTI (Sridhar et al., 2002). UTI patients are susceptible to cause emphysematous cystitis, pyelonephritis, renal or perinephric abscess, bacteremia, and renal papillary necrosis. Bacteraemic patients have more chance to develop acute renal failure (Ronald and Ludwig, 2001).The gram-negative bacteria play an important role in UTI and the most common causative agent is *Escherichia coli* being found in 75–90% cases (Karlowsky et al., 2002; Nakhjavani et al., 2007).The other bacterial uropathogens are *klebsiella* sp., *Proteus mirabilis*, *Pseudomonasaeruginosa*,*Enterococcus* and coagulase negative *Staphylococci* (Omigieet al., 2009).

The bacterial resistance to antibiotic is significant to medical industry and global threat in the treatment of urinary tract infections (UTIs) and hence a constant topic of discussion across the world. It greatly alarmed that the incidence of multidrug resistance (MDR) development and infection are increasing in UTIs (Shahi and Kumar, 2016). Uropathogens are one of the most important MDR bacteria and it developed resistance against almost all commercially available antibiotics including Ampicilin, amoxicillin, ciproflaxacin and all third generation cephalosporin due to the co-expressed resistance mechanisms that lead to MDR effect in these pathogens.Treatment of such MDR is also a major topic of concern since the pathogens are almost resistant to current antibiotics and they easily develop resistance to the new drugs also (Rajivgandhi et al., 2014; Rajivgandhi et al., 2018). In particular, Gram negative bacteria play a vital role in UTIs mainly due to synthesizing some enzymes, efflux pump, and target site modification (Kelly et al., 2016). Recently MDR in these bacterial uropathogens is found toincrease significantly and they show resistance to at least one drug commonly used for eradication and few are resistant to all approved antibiotics (Paterson, 2007). It causes recurrent infections (40-75%) in UTIs due to improper usage, un prescribed format of antibiotics, over dosage and long-term treatment in human, agro farms and veterinary (Moges et al., 2014).

Biofilm development can be considered as a virulence determinant responsible for the long-lasting persistence of bacteria in the genitourinary tract. Urinary catheters and any other prosthetic devices predispose to UTI by destroying natural barriers and providing a site for infection by serving as a substrate for biofilm formation. Fundamental research studies have

demonstrated that biofilm cells are more resistant to antimicrobial agents than planktonic bacterial cells (Tabibian et al., 2008). Reduced antibiotic susceptibility of biofilms contributes to the persistence of infections, such as those associated with implanted devices. Poor antibiotic penetration, nutrient limitation, slow growth, and the formation of persistent cells are hypothesized to constitute multi-layered biofilm (Stewart, 2002). Conventional antimicrobials are not effective against biofilms, and there are relatively few novel compounds or strategies under development or under clinical testing. Increased knowledge regarding the formation of biofilms has led to identification of several possible points for targeted antibiofilm approaches (Flemming and Wingender, 2010; Römling and Balsalobre, 2012).

Actinomycetes are Gram-positive bacteria that have a high percentage of guanine and cytocine in the DNA and are very widespread in nature. The majority of actinomycetes are free living, saprophytic bacteria, widely distributed in soil, water and colonizing plants, and are even able to live in extreme conditions (Nakade, 2012). The genus *Streptomyces* is the most productive group of actinomycetes, because it can produce many antibiotics and other classes of biologically active secondary metabolites (Hozzein et al., 2011). They cover around 80% of total antibiotic products, with other genera trailing numerically. Some examples of secondary metabolites produced by the *Streptomyces* group include erythromycin, tetracycline, streptomycin, chloramphenicol, neomycin, nystatin, amphotricin, kanamycin and cycloheximide, which have an important role in the field of medicine (Hozzein et al., 2011). Some reports have shown that actinomycetes have the capability to inhibit and reduce the biofilm formation of *V. Cholerae* (You et al., 2007) and *Vibrio* spp. (Nithya and Pandian, 2010). *Streptomyces* sp. BFI 230 and *Klebsiasp*. BFI 1562 also have been reported to have antibiofilm activity against a *P. aeruginosa* biofilm (Kim et al., 2009). Keeping all these aspects in mind, the present study is planned wih objectives to study and characterize bioactive compounds from actinomycetes as a antibiofilm agent against multidrug resistant and biofilm forming uropathogens and provide a new alternative treatment for biofilm-associated disease.

3. Review of literature

Urinary tract infections (UTIs) are serious health affecting problems worldwide. *E.coli* accounts for approximately 85% of community acquired UTIs and 50% of hospital acquired UTIs (Ramakant k et.al, 2011). According to (Iqbal et al, 2010), Urinary catheters destroy natural barriers and provide a nidus for infection by serving as a substrate for biofilm formation. Several studies have demonstrated that biofilm cells are more resistant to

antimicrobial agents than planktonic bacterial cells (Tabibian J.H et.al, 2008). The resistance of biofilms to antibiotics contributes to the persistence of infections, such as those associated with implanted devices .Bacterial biofilms play an important role in UTIs, responsible for persistent infections leading to recurrences and relapses (Waturangi. et al, 2016). Approximately 40% of women have had a UTI at some time in their lives (Kunin CM, 2014). *Escherichia coli* (*E. coli*) is the most frequent agent (about 80%) of UTIs in humans and one of the most common causes of Gram-negative nosocomial infections. Further, other bacteria such as *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonasaeruginosa*,*Enterococcus* spp., *Enterobacter* spp, group B *Streptococcus,* and *Staphylococcus* saprophyticus are also involved (Foxman B, 2002).

Eradication ofbacterial biofilms is difficult due to the high level of antimicrobial resistance showed by these structures. Compounds with antibiofilm properties necessary in order to prevent the formation and disrupt the development of biofilm. The ideal situation is a compound or a group of compounds that present not only antibiofilm activity but also the capacity to eradicate multidrug-resistant bacteria. It is also important to study the antibiofilm activity of new combinations of therapeutic strategies including new ones and old antibiotics. Virulence factors should be considered important targets for developing antivirulence compounds that may be applied in the prevention and treatment of infectious diseases.(Sara M. Soto, 2014).Antibiofilm substances from actinomycetes carried various characteristics including lethal bioactive compounds, non-antibacterial active agents and proteolytic activity against *E.coli* and *S. aureus* biofilm formation. It is known that actinomycetes are important sources of natural products and are also potent sources of novel anti-biofilm substances(Kantinanleetanasaksakul and ArinthipThamchaienet).Some actinomycete species were reported to produce *S. aureus*biofilm inhibitors. Methanol extracts of coral-associated *Streptomycesakiyoshiensis*(Bakkiyaraj& Pandian, 2010), extracellular proteases from *Streptomyces sp.* BFI 250 and *Kribbellasp*. BFI 1562 (Kim et al, 2009), alnumycin D from *Streptomyces albus*, granaticin B from *Streptomyces violaceoruber*(Oja et al.), and streptorubin B from *Streptomyces sp*. MC11204 (Suzuki et al.) were shown to inhibit biofilm formation and also disrupt pre-established *S. aureus*biofilms.

The *Streptomyces sp*. showed antimicrobial activity against UTI pathogens. The MIC values of the antimicrobial agent against the test pathogen indicated a potent activity and as such could be used in the generation of new antimicrobial agents. The findings of this study illustrate

that naturally occurring forest actinomycetes have a great potential in the production of antimicrobial agents which could aid in discovery of new antibiotics. This could be of great importance especially in their use to modify urinary catheters to prevent the spread of nosocomial urinary tract infections.Actinomycetes bacteria produce about 70% of the antibiotics that exist. These have many effects including antibacterial, antifungal, antiviral, antiprotozoal properties (AngimaBichang'a Kingsley et al, 2017)

4. **Objectives of Research**
    - Isolation of actinomycetes from diverse ecosystem.
    - Isolation and antibiotic susceptibility testing of uropathogens.
    - Evaluation of biofilm formation potential of uropathogens.
    - Screening and evaluation of bioactive metabolite producing actinomycetesfor antimicrobial and antibiofilm activities against uropathogens.
    - Identification of promising isolate at molecular level.
    - Production and optimization of bioactive compound.
    - Characterization of bioactive compound.
    - Quantification of antibiofilm activity of bioactive compound.
5. **Methodology to be adopted**
    1. Isolation of actinomycetes from different environmental samples will be carried out by using serial dilution methods and selective medias.
    2. Uropathogens will be isolated from sample collected from patients with history of UTI.
    3. Testing drug resistance pattern among uropathogens will be studied using standard and commercially available antibiotics used for treatment of UTI. Agar well diffusion or disc diffusion methods will be used.
    4. Biofilm formation potential of uropathogens will be determined using standard methods such as congo red assay and microtitre plate methods.
    5. Screening of actinomycetes for antibacterial activity against drug resistant uropathogens using agar well diffusion or broth dilution methods.
    6. Screening of actinomycetes for antibiofilm activity against drug resistant uropathogens using standard methods.

7. Molecular identification of potential isolate will be carried out using 16s rRNA sequencing method.
8. Production of bioactive metabolite from selected isolate will be carried out in optimized media.
9. Purification of bioactive metabolite from selected isolate will be carried out using standard methods.
10. Characterization of bioactive metabolite from selected isolate will be carried out using standard analytical methods.

6. Importance of Study

International status:

(PramesitaPrawadikaapsari et al.) reported thatactenomycetes isolate extracts proved to have a broad spectrum of antibiofilm activity because all of extracts were able to inhibit and disperse the biofilm formation of Gram-positive pathogens (*S. aureus*, *S. pneumoniae*, *E. faecalis*) and Gram-negative pathogens (*V. cholerae*, *V. parahaemolyticus*, *P.aeruginosa*) without inhibiting bacterial growth.

National status

(AngimaBichang'a Kingsley et al.)focus on isolation of potent antibiotic producing actinobacteria from rhizosphere and grassland of forest soil samples. Some isolates showed activity against at least one pathogen in primary screening of the isolated pure strains, one actinomycete culture, *Streptomyces sp*. ABK 07 was found as producing a wide spectrum of antimicrobial activity.

7. Proposed work Plan/ Formulation and Structure of Study:

Year-wise Plan of work and targets to be achieved

First year (2021-2022):-

- Completion of course work and Literature survey.
- Sample collection.
- Isolation of Actinomycetes.
- Isolation of Uropathogens.

Second year (2022-2023): -

-Screening of isolated actinomycetesfor antimicrobial andantibiofilm activity.

- Testing drug resistance and biofilm formation potential of uropathogens.

-Production of bioactive compound from selected isolate.

Third year (2023-2024): -

-Purification and Characterization of produced bioactive compound.

-Studies on antimicrobial and antibiofilm activities of actinomycetes.

-Preparation and submission of research article to the reputed national and international journal.

-Compilation and analysis of data.

-Submission of thesis to the university.

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Research guide

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